Absence of Epistasis for Grain Yield in Elite Maize Hybrids

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ABSTRACT

Certain maize (*Zea mays* L.) inbred lines are more successful than others in forming elite hybrids. This study was conducted to determine whether epistatic interactions play a significant role in hybrid performance. Statistical epistasis was measured with a modified generation means model using testcrosses. Six progeny generations (P_1, P_2, F_1, F_2, and a backcross from the F_1 to each parent) were produced for all possible hybrids of a five-parent diallel in both the Iowa Stiff Stalk Synthetic (BSSS) and non-BSSS heterotic groups. Two testers were hybridized to each of the 10 possible hybrid progeny sets in both groups. Each testcross progeny set was evaluated in 10 environments. The nonepistatic model accounted for a large amount of the variation in generation means and fit the data well. Of the 40 maize testcross progeny sets studied, five resulted in a significant epistatic effect for grain yield. Four of the significant epistatic effects showed evidence of linkage, while one was due to unlinked epistatic effects. Our results suggest that parents in a hybrid cross need to be significantly different and testers need to bring out those differences to detect epistasis better by means of testcross generation means.

S TATISTICAL EPISTASIS describes the deviation that occurs when the combined additive effect of two or more genes does not explain an observed phenotype (Falconer and Mackay, 1996). The main focus of statistical epistasis is determining whether or not the phenotype of a given genotype can be predicted by simply adding the effects of its component alleles (Cheverud and Routman, 1995; Phillips, 1998). The difference between the observed phenotype and predictions based on genotype is termed the epistatic deviation. Statistical epistasis differs from the Mendelian view of epistasis where the phenotype is a result of one gene masking the effects of another. Genetic (physiological) epistasis is a genotypic phenomenon, whereas statistical epistasis is both a genetic and population phenomenon based on allele frequencies (Cheverud and Routman, 1995).

Favorable epistatic deviations may become fixed and maintained in inbred lines (Lamkey et al., 1995). These epistatic effects could explain why certain inbreds are more successful than others in forming hybrids, and this knowledge can be beneficial when researchers set up breeding programs (Hallauer and Miranda, 1988; Lamkey et al., 1995). The detection of a high incidence of epistasis among hybrids would suggest that hybrid breeding programs select for epistatic effects.

Measures of epistasis in maize hybrids have been estimated by (i) triple-test crosses, (ii) making comparisons of single, three-way, and double cross hybrids, or (iii) measuring variance components (Hallauer and Miranda, 1988). Epistasis has been measured by generation means analysis (Hallauer and Miranda, 1988) making it possible to detect epistatic effects on the basis of means—a more powerful test than examining variance components (Fenster et al., 1997). The original generation means analysis proposed by Hayman (1958) measured the different generations derived from a cross between two pure lines. Melchinger (1987) proposed testcrossing the generations from Hayman's analysis to an inbred tester, which removes dominance effects from the model that tended to overwhelm the epistatic effects. The gene effects estimated in Melchinger's model are in reference to the F_2 testcross populations versus the F_2 population per se in Hayman's model.

We used the analysis developed by Melchinger (1987) for testcross means of a cross between two inbred lines, their F_1, F_2, and backcross generations. This is a continuation of the work of Lamkey et al. (1995) who first attempted to measure epistatic effects in North American maize germplasm using testcrosses in a generation means analysis. Our aim was to extend the research of Lamkey et al. (1995) to a greater range of U.S. maize germplasm to get a broader view of the importance of epistasis. The significance of the findings reported by Lamkey et al. (1995) suggested that epistasis might play a significant role in many other elite maize hybrids. An experiment designed to test this hypothesis was consequently initiated. An advantage of our experiment compared with previous studies of epistasis is that we have evaluated a large number of hybrid combinations in a single experiment.

The objectives of our research were (i) to estimate genetic means and effects when Melchinger's (1987) Model 1 and Model 2 are applied to testcross progeny sets from a wide selection of maize hybrids, (ii) to determine whether epistasis is present and influencing phenotypic variation, (iii) to clarify which model best explains the variation in the data collected, and (iv) to establish whether these models are useful in detecting epistasis in U.S. elite maize hybrids.

MATERIALS AND METHODS

Genetic Materials

The parental lines in this experiment included yellow dent maize inbreds derived from recurrent selection programs in Iowa Stiff Stalk Synthetic (BSSS) and non-BSSS heterotic groups. The BSSS inbred lines included B14A, B37 (Russell et al., 1971), B73 (Russell, 1972), B84 (Russell, 1979), and B94 (Russell, 1991). The non-BSSS inbred lines included B90, B91 (Russell, 1989), B95 (Hallauer et al., 1992), B97 (Hallauer et al., 1994), and B99 (Hallauer et al., 1995). The five lines

within each heterotic group were crossed in a 5×5 diallel mating design to produce 10 F_1 hybrids for each group. Six generations of progeny were obtained from each of the 20 hybrids: the two parental generations (P_1 and P_2), the F_1, the F_2, and the two generations of the F_1 backcrossed to each parent (BCP_1 and BCP_2). Random plants of the F_1 hybrid were self-pollinated to form the F_2. Those same F_1 hybrid plants were also crossed to P_1 and P_2 plants to make the BCP_1 and BCP_2 generations, respectively. Each group of six progeny generations resulting from a hybrid cross will be referred to as a hybrid progeny set. There are 10 hybrid progeny sets for both the BSSS group and for the non-BSSS group.

Testcrosses of each hybrid progeny set will be referred to as testcross progeny sets. The two testers for the non-BSSS hybrid progeny sets were B104 and B73. B104 was derived from BS13, a population formed from the BSSS heterotic group (Hallauer et al., 1997). Inbred B73 was derived from an advanced recurrent selection population (Cycle 5) of BSSS (Russell, 1972). The two testers for the BSSS hybrid progeny sets were B97 and B112. B97 was selected from Cycle 9 of a reciprocal recurrent selection program in Iowa Corn Borer Synthetic No. 1 (BSCB1) (Hallauer et al., 1994). B112 was selected from Cycle 11 of the BSCB1 population (A.R. Hallauer, personal communication, 2001). The 10 hybrid progeny sets from each heterotic group were crossed to two testers from the opposite heterotic group producing 40 unique testcross progeny sets. Inbred lines were labeled arbitrarily as P_1 or P_2 in a cross generally with the earliest released line within the hybrid pair designated as P_1. An inbred line will always be P_1 or P_2 within hybrids but may be labeled P_1 in one hybrid and P_2 in another. The label for a testcross progeny set followed the form: ($P_1 \times P_2$) * tester.

Adequate seed was not available for testcrossing after producing the backcross generation of (B37 $\times$ B84). These two entries were replaced with filler plots in the field evaluation, and their phenotypic data were removed from the analysis.

Field Evaluation

The 240 entries were evaluated in a 12×20 row-column lattice [$\alpha(0,1)$] experimental design with two replications at five locations for two years. Testcrosses were evaluated at Ames, Carroll, Crawfordsville, Fairfield, and Rippey, IA, in 1999 and 2000. Experimental plots consisted of two rows, 5.49 m long with 0.76 m between rows. Data collected on plots included silking date (days after planting when 50% of the plants in a plot showed visible silks), ear height (cm), plant height (cm), root lodging (percentage of plants leaning greater than 30° from vertical), stalk lodging (percentage of plants with stalks broken at or below the highest ear), machine harvestable grain yield adjusted to 155 g kg^{-1} grain moisture (Mg ha^{-1}), and grain moisture concentration at harvest (g kg^{-1}). The results presented here focus primarily on the effects of epistasis on grain yield across the 40 testcross progeny sets.

Statistical Analysis

Individual environments were analyzed by a mixed-model lattice analysis where rows and columns were fit as random effects and entries were fit as fixed effects. Residuals from these analyses were used to test for normality and outliers. The raw data, corrected for outliers, was used to compute the combined analysis, where the entry $\times$ environment interaction along with rows and columns were fit as random effects. Entries and environments were fit as fixed effects. The variance of the combined entry means was calculated by dividing the

average of the variance of the difference between all possible treatment pairs by two.

The entries and entries $\times$ environment sum of squares were further partitioned into effects due to heterotic groups, generations, testers within heterotic groups, and hybrid progeny sets within heterotic groups. The effects of tester, generation, and the tester $\times$ generation interaction were fit for the 10 hybrid progeny sets from both BSSS and non-BSSS heterotic groups.

The generation means for each testcross progeny set combined over environments were used to fit Melchinger's (1987) Model 1 and Model 2. Each parent inbred line crossed with a tester was included four times in the experiment because each inbred was involved in four F_1 hybrids in the 5×5 diallel. To get a better estimate of these points, each of the four parental testcrosses were averaged together.

Under the null hypothesis of no epistasis, we expect a linear relationship due solely to additive effects [d^T] to explain the differences among testcross generation means. A significant additive effect indicates that genetic differences are present among generations within a testcross progeny set. The alternative hypothesis suggests this relationship deviates from linearity because of combined epistatic (nonadditive) effects [i^T] to give a quadratic function. A significant epistatic effect means that additive effects alone cannot explain the variation present among generations. The superscript T in the following formulas indicates that these values pertain to testcross effects. Therefore, any observable effects within generations of a testcross progeny set are due solely to the original parents. Testcross effects are evident when comparing means of hybrid progeny sets crossed to different testers. Model 1 does not account for epistasis:

$$Y = m^T + x(d^T);$$

where Y = testcross mean of the generation considered; m^T = testcross mean of the F_2 base population in gametic equilibrium; x = coefficient that reflects the percentage of a parent present in each generation relative to the F_2 population: generation P_1 $x = 1$; generation P_2 $x = -1$; generation $F_2 = F_1$ $x = 0$; generation BC_1 $x = 0.5$; and generation BC_2 $x = -0.5$.

$$(d^T) = \Sigma_j \theta_j d_j^T;$$

θ_j = allelic state at locus j (e.g., $+1$ if P_1 contains the favorable allele at locus j and -1 if P_1 contains the unfavorable allele at locus j); and d_j^T = one-half the average effect of a gene substitution at locus j based on the F_2 testcross population.

Model 2 allows for digenic epistasis:

$$Y = m^T + x(d^T) + x^2(i^T);$$

where $Y, m^T, x, (d^T)$, and θ defined as above; $(i^T) = \Sigma_{j<k} \theta_j \theta_k i_{jk}^T$; and i_{jk}^T = additive-by-additive epistatic effect between loci j and k.

The genetic expectations for each generation under Model 1 and 2 were given by Lamkey et al. (1995). The genetic parameters for both models were estimated using weighted least squares:

$$\hat{\beta} = (\mathbf{X'WX})^{-1}(\mathbf{X'Wy});$$

where $\hat{\beta}$ = column vector of estimated genetic parameters; $\mathbf{X}$ = a matrix with elements that are a function of the generation; $\mathbf{W}$ = a matrix with the inverse of the variances of the generation means on the diagonal and zero on the off-diagonal; and $\mathbf{y}$ = column vector of testcross means.

Weighted estimates were calculated because the parental generations are known with more precision than the remaining generations (Mather and Jinks, 1971). Standard errors for the

genetic parameters were calculated as the square root of the diagonal of the $(\mathbf{X'WX})^{-1}$ matrix. A coefficient of multiple determination (R^2) was obtained to explain the amount of variation accounted for by each model. The goodness-of-fit of each model was tested with a weighted Chi-square test as described by Mather and Jinks (1971):

$$\chi^2 = \Sigma[(O - E)^2 \times V];$$

where O = observed testcross generation mean; E = expected testcross generation mean; and V = the inverse of the variance of the testcross generation mean.

RESULTS AND DISCUSSION

The highest mean grain yield (9.59 Mg ha^{-1}) was harvested from the Ames location in 1999, while the lowest mean grain yield (6.04 Mg ha^{-1}) was produced at Crawfordsville in 2000. The 1999 environments combined had the highest overall mean grain yield (8.14 Mg ha^{-1}), grain moisture (222 g kg^{-1}), and root lodging (9.5%). Overall stalk lodging (38.6%), plant height (273 cm), and ear height (135 cm) means were highest in the 2000 locations. Root and stalk lodging rates varied across environments and may have affected yield measurements. The genotype × environment interaction was significant for yield.

Testcrosses with non-BSSS parents (B73 and B104 testers) averaged over all environments tended to have the highest grain yield (7.70 Mg ha^{-1}). B73 testcrosses generally yielded more than all other testcrosses except in 2000 at the Crawfordsville (5.95 Mg ha^{-1}) and Rippey (6.37 Mg ha^{-1}) locations. B112 testcrosses had the lowest yields at all locations except Crawfordsville (8.72 Mg ha^{-1}) and Fairfield (6.95 Mg ha^{-1}) in 1999 and Crawfordsville (6.12 Mg ha^{-1}) and Rippey (6.13 Mg ha^{-1}) in 2000.

Six of the 10 testcross progeny sets from B73 had significant parental differences while only one testcross progeny set from B104 showed a difference between P_1 and P_2 (Fig. 1–Fig. 2). In contrast, these differences were more frequent among BSSS lines crossed to B97 and B112 (Fig. 3–Fig. 4). P_1 differed from P_2 in eight testcross progeny sets of both the B97 and B112 testcrosses. When the hybrid progeny sets are averaged over testers, six sets of BSSS parents had significant tester effects, generation effects, or a combination of both. A tester effect was significant in one out of the 10 sets of non-BSSS parents. The tester × generation effect was nonsignificant in all cases.

The parental lines and testers used in this study were chosen as a combination of the best and most recently developed inbreds from recurrent selection programs in BSSS and non-BSSS when this experiment was initiated. The same testers were used to evaluate the potential of the parental lines before they were released. To that end, those evaluations were based on the general combining ability of the lines—how well they perform averaged over several testers (Hallauer and Miranda, 1988). Individual tester effects—specific combining ability—may not have been as desirable as overall performance.

Melchinger's (1987) Model 1 provides the expectations for a trait not affected by epistasis. Variation for

significant additive effects [d^T] was observed among testers. B97 and B112 testcrosses had eight and nine testcross progeny sets, respectively, with significant additive effects indicating differences among generation means (Table 1). B73 and B104 testcrosses had fewer testcross progeny sets with significant additive effects (Table 2). Six B73 testcross progeny sets had significant additive effects. The B104 testcrosses were unique because nine out of 10 additive effects were not significant, thus indicating that B104 may be masking differences among inbreds.

Melchinger's (1987) Model 2 includes an epistatic term [i^T] in addition to the main and additive effect terms included in Model 1. This term is a measure of unlinked additive-by-additive epistasis. Only one testcross progeny set [(B90 × B95)*B104] had a significant ($P \leq 0.05$) epistatic component (0.37 ± 0.18) (Table 2). This set was unusual because its additive effect was not significant. There was, however, a significant difference between P and the F_2 for this set. This was depicted as a parabolic relationship (Fig. 2b).

The analysis of hybrid progeny sets averaged across both testers for a given heterotic group brought out another instance for unlinked epistasis in (B37 × B73) (Table 1). An average across B73 and B104 testers did not reveal new cases for epistasis (Table 2).

Before making further comparisons, we will consider the relationship between the F_1 and the F_2. The F_1 and F_2 have the same gametic array when considering the population as a whole. Therefore, these generations are expected to have the same mean values with epistasis and no linkage (Melchinger, 1987). When the F_1 and F_2 testcross means did differ and there was a nonsignificant epistatic effect, the observed differences in means are due to linked epistatic effects (Melchinger, 1987). There were four instances where the F_1 and F_2 testcross means differed [(B91 × B99)*B104; (B97 × B99)*B104; (B14A × B84)*B97; and (B14A × B73)*B112], and these sets did not have significant epistatic effects (Tables 1–2). Therefore, there were four cases for linked epistatic effects.

Given the original experimental test of the epistatic model (Melchinger et al., 1988) and a subsequent study (Lamkey et al., 1995) were evaluated without the F_1, we removed it from our data set to determine whether inclusion of this generation may have affected our ability to detect significant genetic parameters. Removing the F_1 did not alter our findings for genetic effects on grain yield. Those effects that were significant remained so and those that were not likewise remained nonsignificant.

How well do the additive effect and additive-by-additive epistatic effect explain the variation in a testcross progeny set? Although epistatic effects per se were rarely significant for grain yield, in certain crosses, including epistasis explained a substantial amount of the variation among generation means. For Model 1 of B104 testcrosses, sums of squares accounted for up to 67% of the variation. In Model 2, a maximum of 85% of the variation was explained. This was in contrast to Model 1 for other testers that accounted for up to 96% of the

Fig. 1. Fitting Model 1 and Model 2 to the generation means for each B73 testcross progeny set. Testcross grain yield (Mg ha^{-1}) is plotted against the percentage of Parent 1 in the cross. Figure captions are in the form: (P$_1$ × P$_2$) * tester. Solid squares = observed grain yield with 95% confidence intervals; dashed line = Model 1 (linkage—no epistasis); solid line = Model 2 (epistasis—no linkage).

Fig. 2. Fitting Model 1 and Model 2 to the generation means in each B104 testcross progeny set. Testcross grain yield (Mg ha^{-1}) is plotted against the percentage of Parent 1 in the cross. Figure captions are in the form: (P$_1$ × P$_2$) * tester. Solid squares = observed grain yield with 95% confidence intervals; dashed line = Model 1 (linkage—no epistasis); solid line = Model 2 (epistasis—no linkage).
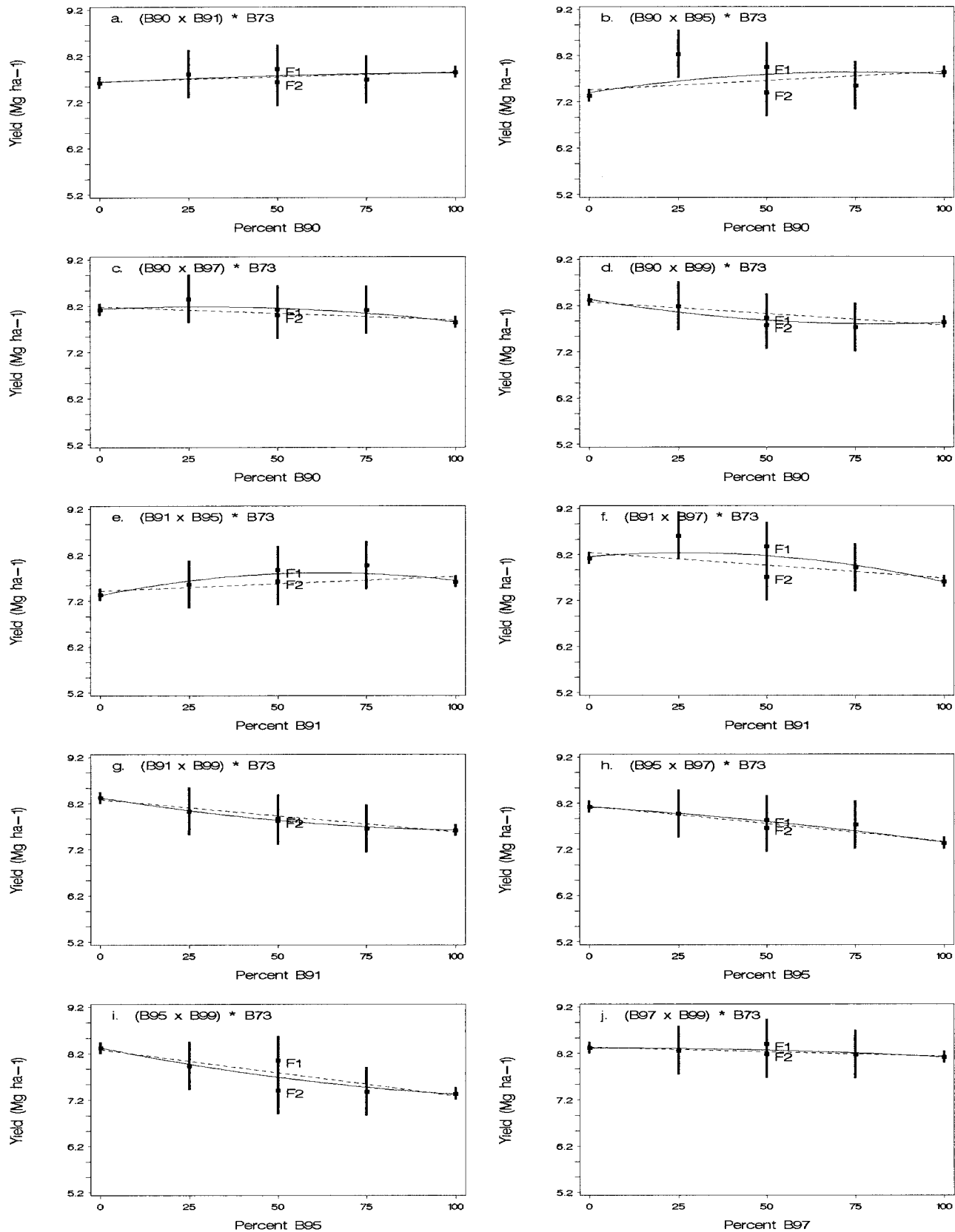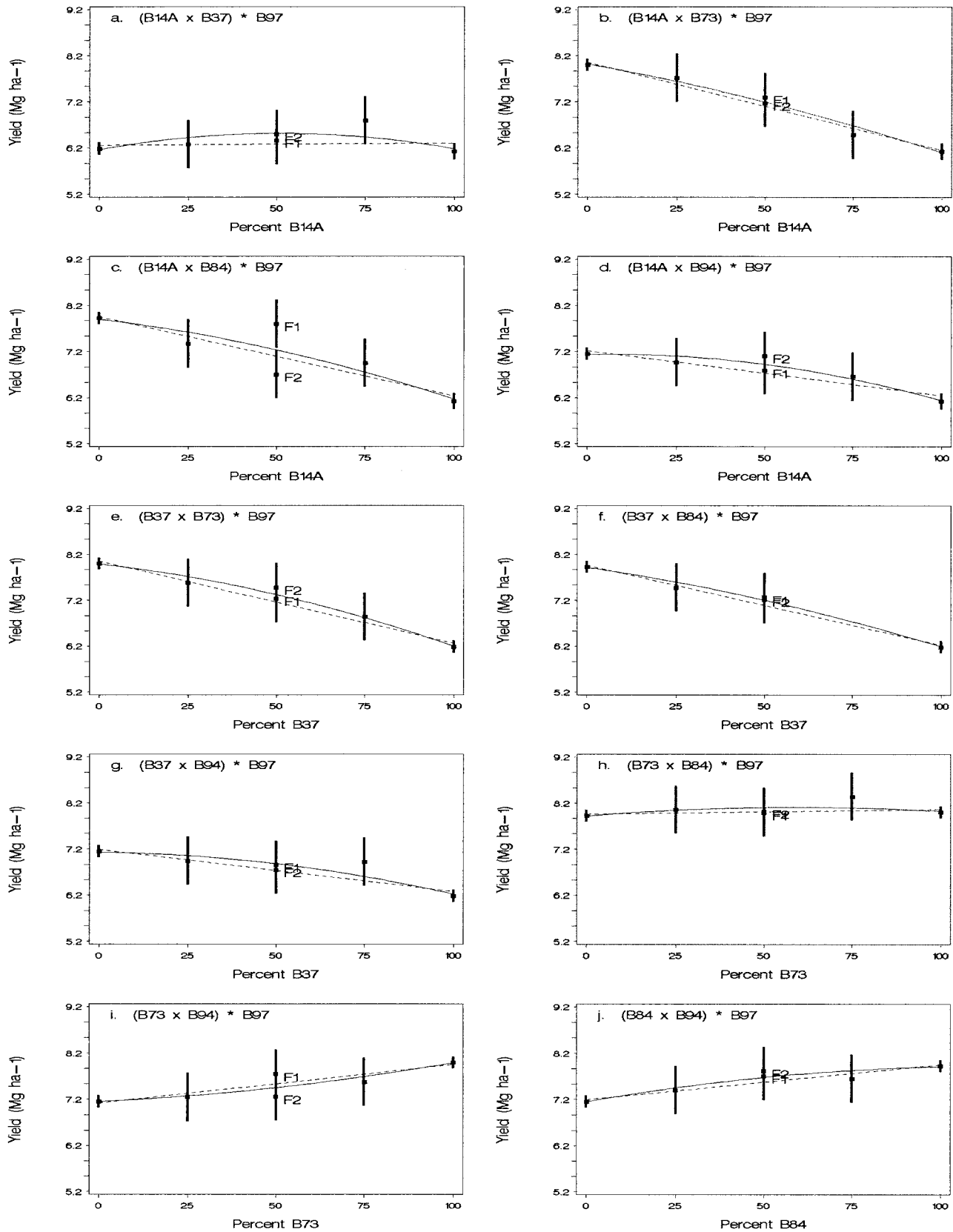
Fig. 3. Fitting Model 1 and Model 2 to the generation means in each B97 testcross progeny set. Testcross grain yield (Mg ha^{-1}) is plotted against the percentage of Parent 1 in the cross. Figure captions are in the form: (P$_1$ × P$_2$) * tester. Solid squares = observed grain yield with 95% confidence intervals; dashed line = Model 1 (linkage—no epistasis); solid line = Model 2 (epistasis—no linkage).
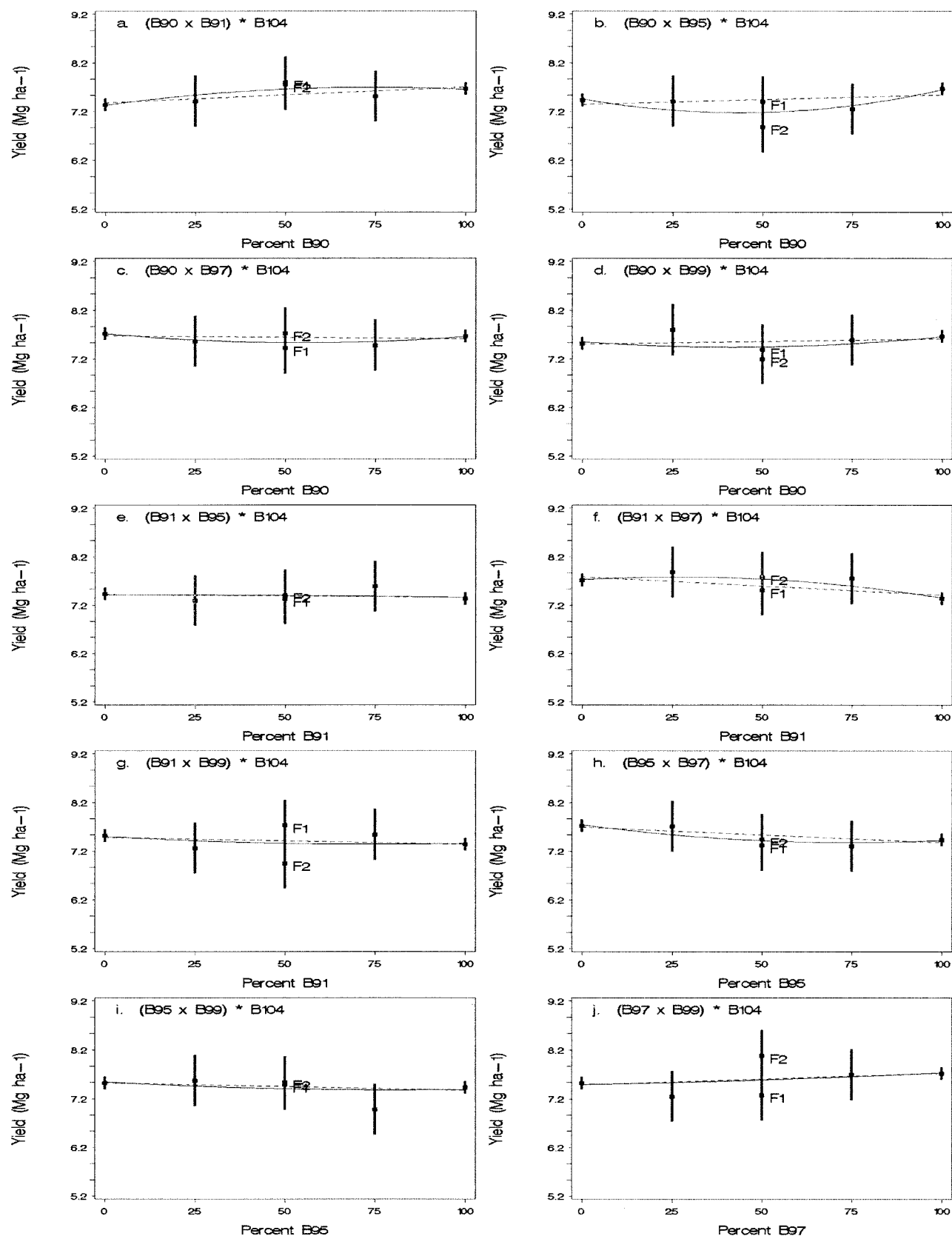
Fig. 4. Fitting Model 1 and Model 2 to the generation means in each B112 testcross progeny set. Testcross grain yield (Mg ha^{-1}) is plotted against the percentage of Parent 1 in the cross. Figure captions are in the form: (P$_1$ × P$_2$) * tester. Solid squares = observed grain yield with 95% confidence intervals; dashed line = Model 1 (linkage—no epistasis); solid line = Model 2 (epistasis—no linkage).
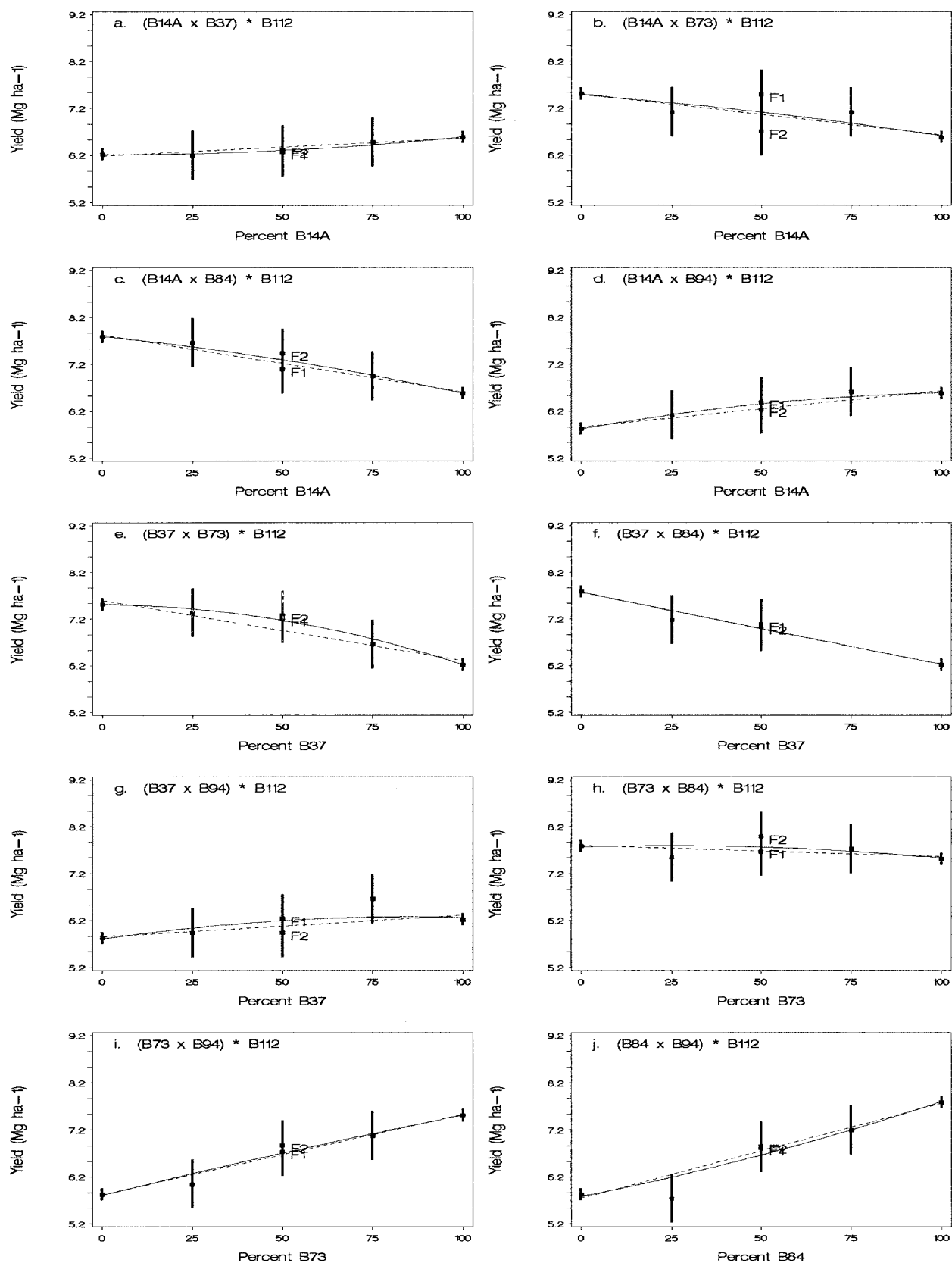
Table 1. Genetic effects for B97, B112, and non-BSSS combined testcross progeny sets. Regression estimates and their standard errors were determined by fitting Model 1 and Model 2 to testcross grain yields evaluated over 10 environments for P_1, BCP_1, F_1, F_2, BCP_2, and P_2 generations.

Grain Yield

	B14A × B37†	B14A × B73	B14A × B84	B14A × B94	B37 × B73	B37 × B84	B37 × B94	B73 × B84	B73 × B94	B84 × B94
					Mg ha⁻¹					
B97 Testcrosses										
Model 1:										
m^T‡	6.28 ± 0.08	7.10 ± 0.08	7.09 ± 0.08	6.73 ± 0.08	7.16 ± 0.08	7.09 ± 0.08	6.74 ± 0.08	8.01 ± 0.08	7.54 ± 0.08	7.57 ± 0.08
d^T	0.02 ± 0.10	-0.95 ± 0.10	-0.86 ± 0.10	-0.49 ± 0.10	-0.90 ± 0.09	-0.87 ± 0.09	-0.46 ± 0.09	0.05 ± 0.09	0.42 ± 0.09	0.38 ± 0.09
χ^2 (4)§	5.88	1.35	11.23*	3.31	2.30	0.86	3.21	1.67	2.37	1.44
R^2 (%)	1.0	98.6	87.3	88.2	97.7	99.0	88.6	16.1	89.8	92.2
Model 2:										
m^T	6.51 ± 0.15	7.19 ± 0.15	7.23 ± 0.15	6.92 ± 0.15	7.32 ± 0.15	7.20 ± 0.17	6.88 ± 0.15	8.10 ± 0.15	7.45 ± 0.15	7.68 ± 0.15
d^T	0.01 ± 0.10	-0.96 ± 0.10	-0.87 ± 0.10	-0.50 ± 0.10	-0.90 ± 0.09	-0.86 ± 0.09	-0.46 ± 0.09	0.05 ± 0.09	0.42 ± 0.09	0.38 ± 0.09
i^T	-0.34 ± 0.19	-0.14 ± 0.19	-0.20 ± 0.19	-0.28 ± 0.19	-0.23 ± 0.18	-0.15 ± 0.19	-0.21 ± 0.18	-0.13 ± 0.18	0.12 ± 0.18	-0.15 ± 0.18
χ^2 (3)	2.57	0.82	10.05*	1.05	0.71	0.27	1.96	1.15	1.96	0.81
R^2 (%)	56.6	99.2	88.6	96.3	99.3	99.7	93.0	42.1	91.6	95.6
B112 Testcrosses										
Model 1:										
m^T	6.37 ± 0.08	7.07 ± 0.08	7.22 ± 0.08	6.25 ± 0.08	6.95 ± 0.08	7.00 ± 0.08	6.08 ± 0.08	7.68 ± 0.08	6.68 ± 0.08	6.76 ± 0.08
d^T	0.19 ± 0.09	-0.44 ± 0.09	-0.61 ± 0.09	0.38 ± 0.09	-0.65 ± 0.09	-0.77 ± 0.09	0.23 ± 0.09	-0.12 ± 0.09	0.85 ± 0.09	1.01 ± 0.09
χ^2 (4)	0.40	5.87	1.30	1.04	3.26	0.76	4.14	2.17	1.23	4.36
R^2 (%)	91.2	79.6	97.2	94.4	93.9	98.9	59.8	43.8	98.6	96.5
Model 2:										
m^T	6.30 ± 0.15	7.12 ± 0.15	7.30 ± 0.15	6.36 ± 0.15	7.17 ± 0.15	6.99 ± 0.17	6.20 ± 0.15	7.76 ± 0.15	6.71 ± 0.15	6.67 ± 0.15
d^T	0.19 ± 0.09	-0.44 ± 0.09	-0.61 ± 0.09	0.38 ± 0.09	-0.65 ± 0.09	-0.77 ± 0.09	0.23 ± 0.09	-0.12 ± 0.09	0.85 ± 0.09	1.01 ± 0.09
i^T	0.10 ± 0.18	-0.07 ± 0.18	-0.11 ± 0.18	-0.15 ± 0.18	-0.31 ± 0.18	0.01 ± 0.19	-0.16 ± 0.18	-0.12 ± 0.18	-0.05 ± 0.18	0.13 ± 0.18
χ^2 (3)	0.09	5.74	0.95	0.39	0.44	0.76	3.36	1.76	1.16	3.89
R^2 (%)	98.1	80.1	97.9	97.9	99.2	98.9	67.4	54.3	98.7	96.9
Non-BSSS Testcrosses										
Model 1:										
m^T	6.32 ± 0.05	7.08 ± 0.05	7.15 ± 0.05	6.49 ± 0.05	7.05 ± 0.05	7.04 ± 0.05	6.41 ± 0.05	7.84 ± 0.05	7.11 ± 0.05	7.17 ± 0.05
d^T	0.09 ± 0.07	-0.70 ± 0.07	-0.74 ± 0.07	-0.06 ± 0.07	-0.77 ± 0.07	-0.82 ± 0.07	-0.11 ± 0.07	-0.03 ± 0.07	0.64 ± 0.07	0.69 ± 0.07
χ^2 (4)	2.71	3.64	4.00	3.23	5.21	1.23	7.15	2.42	1.44	3.14
R^2 (%)	43.9	97.0	97.0	20.4	96.5	99.2	30.6	10.0	98.5	97.3
Model 2:										
m^T	6.41 ± 0.11	7.15 ± 0.11	7.27 ± 0.11	6.64 ± 0.11	7.25 ± 0.11	7.10 ± 0.11	6.54 ± 0.11	7.93 ± 0.11	7.08 ± 0.11	7.17 ± 0.11
d^T	0.09 ± 0.07	-0.70 ± 0.07	-0.74 ± 0.07	-0.06 ± 0.07	-0.77 ± 0.07	-0.82 ± 0.07	-0.11 ± 0.07	-0.03 ± 0.07	0.64 ± 0.07	0.69 ± 0.07
i^T	-0.12 ± 0.13	-0.10 ± 0.13	-0.16 ± 0.13	-0.22 ± 0.13	-0.27 ± 0.13	-0.07 ± 0.13	-0.18 ± 0.13	-0.12 ± 0.13	0.04 ± 0.13	-0.01 ± 0.13
χ^2 (3)	1.81	3.04	2.50	0.45	0.89	0.96	5.15	1.50	1.36	3.13
R^2 (%)	62.6	97.5	98.2	89.0	99.4	99.4	50.0	44.1	98.6	97.4

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† Testcross progeny sets are in the form: $P_1 \times P_2$.
‡ For definition of genetic effects, see Statistical Analysis section in Materials and Methods.
§ Chi-square degrees of freedom in parentheses.

Table 2. Genetic effects for B73, B104, and BSSS combined testcross progeny sets. Regression estimates and their standard errors were determined by fitting Model 1 and Model 2 to testcross grain yields evaluated over 10 environments for P_1, BCP_1, F_1, BCP_2, F_2, and P_2 generations.

	B90 × B91†	B90 × B95	B90 × B97	B90 × B99	B91 × B95	B91 × B97	B91 × B99	B95 × B97	B95 × B99	B97 × B99
					Grain Yield ($Mg\ ha^{-1}$)					
B73 Testcrosses										
Model 1:										
m^T‡	7.74 ± 0.08	7.66 ± 0.08	8.04 ± 0.08	8.03 ± 0.08	7.57 ± 0.08	7.97 ± 0.08	7.93 ± 0.08	7.75 ± 0.08	7.79 ± 0.08	8.24 ± 0.08
d^T	0.10 ± 0.09	0.20 ± 0.09	−0.14 ± 0.09	−0.25 ± 0.09	0.16 ± 0.09	−0.28 ± 0.09	−0.35 ± 0.09	−0.38 ± 0.09	−0.50 ± 0.09	−0.10 ± 0.09
χ^2 (4)§	1.00	10.02*	1.54	1.80	3.82	8.09	0.60	0.71	3.74	0.49
R^2 (%)	55.0	32.4	61.6	80.7	43.2	53.4	96.1	96.1	88.7	70.6
Model 2:										
m^T	7.77 ± 0.15	7.79 ± 0.15	8.15 ± 0.15	7.88 ± 0.15	7.79 ± 0.15	8.17 ± 0.15	7.83 ± 0.15	7.80 ± 0.15	7.69 ± 0.15	8.27 ± 0.15
d^T	0.10 ± 0.09	0.20 ± 0.09	−0.14 ± 0.09	−0.25 ± 0.09	0.16 ± 0.09	−0.28 ± 0.09	−0.35 ± 0.09	−0.38 ± 0.09	−0.50 ± 0.09	−0.10 ± 0.09
i^T	−0.04 ± 0.18	−0.19 ± 0.18	−0.16 ± 0.18	0.21 ± 0.18	−0.31 ± 0.18	−0.29 ± 0.18	0.14 ± 0.18	−0.07 ± 0.18	0.14 ± 0.18	−0.05 ± 0.18
χ^2 (3)	0.96	8.96*	0.81	0.54	1.06	5.54	0.04	0.58	3.18	0.41
R^2 (%)	56.8	39.5	79.9	94.2	84.3	68.1	99.7	96.8	90.4	75.3
B104 Testcrosses										
Model 1:										
m^T	7.54 ± 0.08	7.45 ± 0.08	7.65 ± 0.08	7.56 ± 0.08	7.39 ± 0.08	7.60 ± 0.08	7.41 ± 0.08	7.53 ± 0.08	7.45 ± 0.08	7.61 ± 0.08
d^T	0.16 ± 0.09	0.10 ± 0.09	−0.03 ± 0.09	0.05 ± 0.09	−0.03 ± 0.09	−0.19 ± 0.09	−0.07 ± 0.09	−0.16 ± 0.09	−0.08 ± 0.09	0.12 ± 0.09
χ^2 (4)	1.95	6.64	1.54	3.40	0.93	2.45	5.39	1.49	3.07	5.98
R^2 (%)	61.1	15.0	7.3	9.5	9.3	63.6	10.0	67.2	18.3	22.4
Model 2:										
m^T	7.66 ± 0.15	7.18 ± 0.15	7.53 ± 0.15	7.45 ± 0.15	7.40 ± 0.15	7.74 ± 0.15	7.36 ± 0.15	7.42 ± 0.15	7.41 ± 0.15	7.59 ± 0.15
d^T	0.16 ± 0.09	0.10 ± 0.09	−0.03 ± 0.09	0.05 ± 0.09	−0.03 ± 0.09	−0.19 ± 0.09	−0.07 ± 0.09	−0.16 ± 0.09	−0.08 ± 0.09	0.12 ± 0.09
i^T	−0.17 ± 0.18	0.37 ± 0.18	0.16 ± 0.18	0.16 ± 0.18	−0.01 ± 0.18	−0.20 ± 0.18	0.07 ± 0.18	0.17 ± 0.18	0.06 ± 0.18	0.03 ± 0.18
χ^2 (3)	1.13	2.54	0.82	2.67	0.93	1.24	5.23	0.67	2.95	5.96
R^2 (%)	77.5	67.5	50.5	29.0	9.7	81.5	12.7	85.2	21.6	22.7
BSSS Testcrosses										
Model 1:										
m^T	7.64 ± 0.05	7.55 ± 0.05	7.84 ± 0.05	7.79 ± 0.05	7.48 ± 0.05	7.78 ± 0.05	7.67 ± 0.05	7.64 ± 0.05	7.62 ± 0.05	7.92 ± 0.05
d^T	0.13 ± 0.07	0.15 ± 0.07	−0.09 ± 0.07	−0.10 ± 0.07	0.06 ± 0.07	−0.23 ± 0.07	−0.21 ± 0.07	−0.27 ± 0.07	−0.29 ± 0.07	0.01 ± 0.07
χ^2 (4)	1.92	10.37*	0.30	4.44	3.08	6.51	3.42	0.49	4.09	2.21
R^2 (%)	68.0	34.0	85.9	34.1	24.1	66.7	75.9	97.3	82.7	1.2
Model 2:										
m^T	7.71 ± 0.11	7.49 ± 0.11	7.84 ± 0.11	7.67 ± 0.11	7.59 ± 0.11	7.96 ± 0.11	7.59 ± 0.11	7.61 ± 0.11	7.55 ± 0.11	7.93 ± 0.11
d^T	0.13 ± 0.07	0.15 ± 0.07	−0.09 ± 0.07	−0.10 ± 0.07	0.06 ± 0.07	−0.23 ± 0.07	−0.21 ± 0.07	−0.27 ± 0.07	−0.29 ± 0.07	0.01 ± 0.07
i^T	−0.10 ± 0.13	0.09 ± 0.13	0.00 ± 0.13	0.18 ± 0.13	−0.16 ± 0.13	−0.25 ± 0.13	0.11 ± 0.13	0.05 ± 0.13	0.10 ± 0.13	−0.01 ± 0.13
χ^2 (3)	1.31	9.87*	0.30	2.49	1.58	2.88	2.76	0.35	3.49	2.20
R^2 (%)	78.2	37.2	85.9	63.1	61.0	85.3	80.6	98.1	85.3	1.6

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

† Testcross progeny sets are in the form: $P_1 \times P_2$.

‡ For definition of genetic effects, see Statistical Analysis section in Materials and Methods.

§ Chi-square degrees of freedom in parentheses.

variation (B73 testcrosses; Table 2) and Model 2 up to 99% of the variation (B112 testcrosses; Table 1). The group of crosses of BSSS lines to non-BSSS testers (B97 and B112) provided the best fit to the epistatic model as they explained a higher amount of variation (R^2) than did B73 and B104 testcrosses.

One prominent exception in the group of B97 and B112 testcrosses involved (B73 × B84), the parental lines studied by Lamkey et al. (1995). Model 2 accounted for the least amount of the variation among the generation means for both the B97 and the B112 testers (42 and 54%, respectively). When studied by Lamkey et al. (1995), this cross was evaluated with the Mo17 tester, and Model 2 explained 69% of the variation among generation means. The Mo17 testcross gave evidence for a classic case of epistasis. The additive effect indicated a distinction among generation means, and unlinked epistasis was detected. In addition, the parents significantly outyielded the backcross and F_2 generations. For the B97 and B112 testcrosses, the values for generation means did not differ, there was no significant epistatic effect, and the parent means overlapped those of the backcross and F_2 generations. This is strong evidence that detection of epistasis appears to be tester dependent (Eta-Ndu and Openshaw, 1999). The parental lines chosen by Lamkey et al. (1995) were crossed to a tester that allowed maximum expression of the genetic differences between progeny generations to obtain a detectable level of epistatic effect.

Implications for Statistical Modeling of Epistasis

The reported experimental design allows for performance comparisons among several lines and their progeny in relation to each other, in combination with different testers, and across heterotic groups. Because of these factors, we have been able to make broader statements regarding epistasis than earlier studies.

Analysis of 40 testcross progeny sets indicated that epistatic effects especially for grain yield were not as prevalent as expected on the basis of previous reports using Melchinger's (1987) testcross generation means models. These results were contrary to our initial expectations. Earlier research suggested that epistasis would be common in testcrosses involving a cross between two related parents (Lamkey et al., 1995). However, in our study, the epistatic effect was rarely significant (Tables 1–2). The large R^2 values for Model 1 combined with the infrequent detection of epistasis suggested a minor role for epistasis.

A better fit to a linear model may suggest no net epistatic effects because presence of both positive and negative epistatic effects could cancel out and produce a linear response (Hallauer and Miranda, 1988). Failure of our epistatic model can also indicate higher order linked or unlinked epistatic interactions (e.g., trigenic epistasis) (Mather and Jinks, 1971) or confounding of some epistatic effects within the measurement for additive effects (Cheverud and Routman, 1995). Averaging testcross means over environments has been observed to have a role in detection of epistasis. For example,

when means were pooled over environments, Martin and Hallauer (1976) observed a decrease in frequency of significant epistatic effects compared to the individual environment analyses.

Choice of parents (Sprague et al., 1962) and testers (Melchinger, 1987) is important for measuring epistasis as well as the genotype × environment combinations studied (Martin and Hallauer, 1976). An enhancement to our study would be to incorporate lines selected expressly for specific combining ability and determine whether we can detect epistasis using that material. Such lines for evaluation could be the parent lines used in commercial maize hybrids. The parents, however, have to be more than just the best lines available; they have to be measurably different when crossed to the same testers. Testing these lines would allow for a direct measure of epistasis and its effect on commercial hybrids.

We know epistasis has a role in phenotype expression (Coe et al., 1988; Avery and Wasserman, 1992), but an appropriate test to estimate it accurately is elusive. Thus, recognizing the inadequacy of current statistical models for estimating epistasis, some suggest, "it is time to move on" to approaches where genotypes are known (Templeton, 2000). Results of marker-assisted studies of quantitative traits clearly show that epistasis plays a role in their inheritance (Yu et al., 1997), as well as in plant growth and development (Li et al., 1997). Approaches like these involve actively searching for epistasis, rather than it being what is left over after all other factors (e.g., additive and dominance effects) have been accounted for (Templeton, 2000).

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